Mycorrhizae : physiology and genetics - Les mycorhizes : physiologie et génétique.
1st ESM/1er SEM, Dijon, 1-5 July 1985.- INRA, Paris, 1986

Utilization of protein by ecto-mycorrhizal fungi and by their host plants

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INTRODUCTION

The lateral roots of most of the tree species which dominate the boreal and temperate forests are sheathed by mycorrhizal fungi (HARLEY & SMITH, 1983), the mycelia of which provide intimate contact between the root surface and the organic horizons of the soil in which these roots are normally concentrated (HARLEY, 1940; MEYER, 1973; PERSSON, 1983). FRANK, (1894), in one of the earliest descriptions of the mycorrhizal symbiosis, proposed that infection might provide access to organic nitrogen compounds which constitute the major fund of this nutrient in forest soils. However, despite the quantitative significance of organic N in these soils and the fact that the major mineral nitrogen source, ammonium, is normally present in extremely low concentrations (COLE, 1981), most investigations of the nitrogen nutrition of ectomycorrhizal plants have examined the assimilation of mineral (MELIN & NILSSON, 1952; CARRODUS, 1967) or simple amino-N (MELIN & NILSSON, 1953; ALEXANDER, 1983) sources, and the possibility that organic polymers could be a significant source of nitrogen for the plant has been largely rejected or overlooked since the time of Frank. The ability of mycorrhizal fungi to utilise pure protein as a nitrogen source has now been investigated by growing the fungi both in pure culture, and in mycorrhizal association with host plants in circumstances where protein was the only source of nitrogen available to the organisms.

MATERIALS AND METHODS

The fungi examined were *Suillus bovinus, Rhizopogon roseolus* and *Pisolithus tinctorius* all of which are ectomycorrhizal species with world-wide distribution, the first two occurring normally in organic soils, the latter on mineral substrates. Their ability to utilize mineral and polymeric organic nitrogen soures was compared first in pure culture and then in mycorrhizal association with the economically important host tree *Pinus contorta*. Ammonium was used as the mineral N source and bovine serum albumin (BSA) - mol. wt. 67,000, N content 16% -was selected as the protein N source because of its purity (99%) and high solubility.

Discs of mycelium of each of the fungi were cut from agar plates and transferred to petri dishes containing 25 mls of a nitrogen and carbon free basal mineral medium (NORKRANS, 1950) supplemented with either $(NH_4)_2SO_4$ or BSA to give a nitrogen concentration of 243 mg l and with glucose to give a C:N ratio of 15:1. The pH of the media were adjusted at the time of inoculation using H_2SO_4 or NaOH to give a starting pH of 5.0 in the case of NH_4^+ and 3, 4, 5, 6 or 7 in the case of solutions containing BSA. Three cultures of each fungus were harvested from each treatment at 20, 40 and 60 days. Dry weight yields and mycelial N contents were determined at each harvest as were pH and residual N contents of the culture media.

To analyse the role of mycorrhizas in the assimilation of protein N, *P. contorta* seedlings were first raised in the mycorrhizal (M) or non-mycorrhizal (NM) condition in 250 ml Erlenmeyer flasks each containing 20 grams of perlite. One batch of flasks was set up for each of the three fungi and one for the NM controls. In order to sustain fungal growth during the mycorrhizal synthesis a small quantity of 'starter' nitrogen was necessary. This was supplied as 1 mg of N per flask in the form of $(NH_4)_2SO_4$ to those M and NM flasks subsequently to receive NH_4^+-N, or, because the presence of NH_4^+-N is known to inhibit protease production, as arginine to those flasks later to receive BSA. In the NM treatment one set of flasks received no starter nitrogen (-N). Nine flasks of each starter treatment (NH_4^+ and Arg) were inoculated at time zero with discs of mycelium of each of the mycorrhizal fungi and all flasks were incubated for 20 d at which time three aseptically germinated pine seedlings were added to all flasks. After a further 40 d, when all seedlings in the M series had become heavily mycorrhizal, plants were harvested from three flasks (9 plants) of every treatment (Harvest 1) and the main nitrogen treatments were commenced. Four mg of N were added either as BSA or $(NH_4)_2SO_4$ to all flasks of the M series and to all of the NM series with the exception of the - N flasks. The latter were sub-divided into two sets, one of which was retained in the - N condition while the other received BSA alone, so that discrimination between the utilization of starter and protein N could be obtained. Three flasks of each of the six treatments viz. M + BSA, M + NH_4, NM + BSA, NM + NH_4, NM-N, NM - starter N + BSA, were harvested after subsequent periods of 30 and 60 d. Plants were divided into roots and shoots which were oven dried, weighed and digested in an H_2SO_4-Na_2SO_4-Se mixture before their total N contents were determined by a modified Nessler procedure. Data from plant dry weight and nitrogen measurements were subjected to a two way analysis of variance to determine the significance of the treatment effects and their interactions. Planned pair-wise comparisons of M and NM categories of plants within individual nitrogen treatments were subsequently carried out using 't' tests.

RESULTS

All three fungi utilised protein as their sole source of nitrogen (Fig. 1a, b, c). Yields were lower than those on ammonium at the first harvest, but thereafter they were as high as that on the mineral nitrogen. *S. bovinus* provided the highest yields while the lowest were obtained from *P. tinctorius*, the proteolytic activity of which was particularly strongly inhibited at pH 6 and 7. Growth of all fungi led to acidification of the medium and in each case the optimum pH for growth and nitrogen utilisation appeared to be within the range 4 to 5. The results suggest that the enzyme responsible for proteolytic activity is an acid protease.

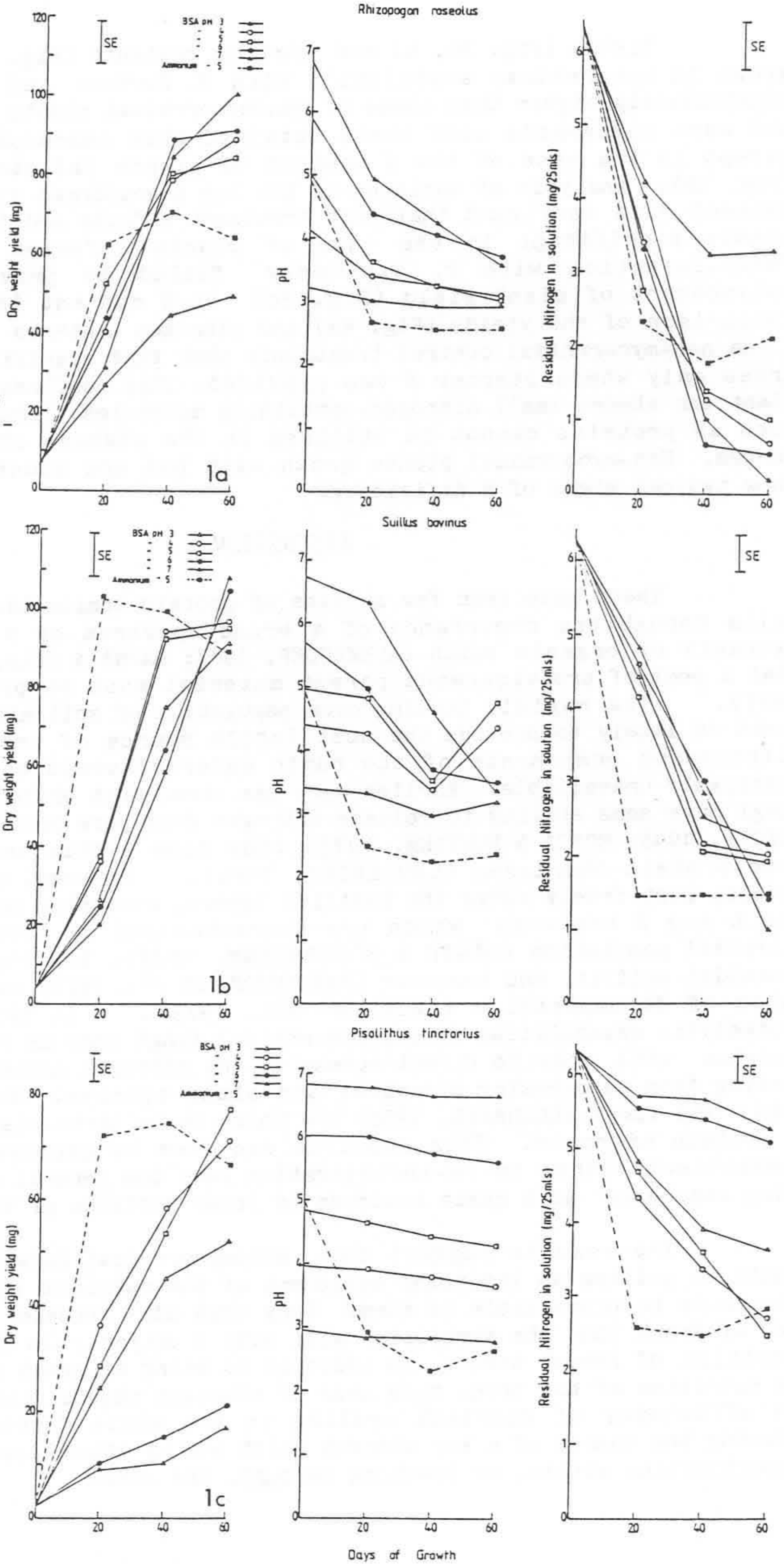

Figure 1. Dry weights, pH changes and residual Nitrogen contents when R. roseolus (Fig. 1a), S. bovinus (Fig. 1b) and P. tinctorius (Fig. 1c) were grown on BSA or NH_4 as sole N source.

Yields (Fig. 2a, b) and nitrogen contents (Fig. 3a, b) of plants grown in mycorrhizal association with *S. bovinus* and *R. roseolus* were significantly higher than those of non-mycorrhizal plants when grown on BSA and were comparable with those obtained when ammonium was the N source except in the case of the N content of plants infected with *S. bovinus* (Fig. 3b). Analysis of variance of the log transformed yield and nitrogen content data confirmed that most treatment effects and interractions were highly significant in the case of plants infected with these two fungi.Infection with *P. tinctorius* failed to provide significant enhancement of plant yield (Fig. 2c) or N content (Fig. 3c) on BSA. Comparison of the yields (Fig. 4a) and nitrogen contents (Fig. 4b) in the three non-mycorrhizal control treatments show that significant differences arose only where starter N was provided. This confirms that whereas the plant can absorb small nitrogen-containing molecules, large polymeric forms such as proteins cannot be utilised in the absence of the mycorrhizal fungus. Non-mycorrhizal plants grown with BSA are stunted (Fig. 5) and show typical signs of N deficiency.

DISCUSSION

There have been few studies of protein composition or turnover in soils though the occurrence of a broad spectrum of α-amino-acids in extracts of organic soils (ALEXANDER, 1983; BAJWA & READ, 1985) indicates that a pool of proteinaceous parent material must be present (BREMNER, 1967). The rapidly turning over population of soil micro-organisms and roots is likely to provide the most labile source of protein, while the nitrogenous components of the humic material would be expected to be relatively unavailable. Earlier work has shown that while ectomycorrhizal fungi have some ability to release nitrogen from pure protein (MELIN, 1925; MIKOLA, 1948; MOSCA & FONTANA, 1975) they have little access to that in humo-protein complexes (LUNDEBERG, 1970). However, mycorrhizal roots develop most freely above the humified layers, occurring predominantly in the L and F horizons which are characterised by having the largest microbial population (BÅÅTH & SÖDERSTRÖM, 1979), the highest levels of microbial activity and turnover (PARKINSON *et al.*, 1978) and the most rapid rates of decomposition (SWIFT *et al.*, 1979). In this position the proteolytic capabilities of ectomycorrhizal fungi such as *S. bovinus* and *R. roseolus* will provide direct access to the nitrogen contained in proteins leaking from decomposing microbial and plant tissues. Bacteria (LURIA, 1960) and fungi (COCHRANE, 1958) are known to be particularly rich sources of protein nitrogen. This resource can thus be captured before it is immobilised either by re-incorporation into the general microflora or by being complexed with humic residues in lower horizons of the soil.

The results suggest that ectomycorrhizal infection will enable plants to utilise an important component of the nutrient fund which would otherwise be unavailable to them. They thus add credence to the original view of Frank that the symbiosis will play a major role in the nitrogen nutrition of forest trees. In addition to being of great significance for the nutrition of the tree, this mode of nitrogen acquisition will increase the efficiency of nutrient cycling in the whole forest ecosystem by reducing the losses of a key element which would otherwise arise through immobilisation within, or leaching through, the soil.

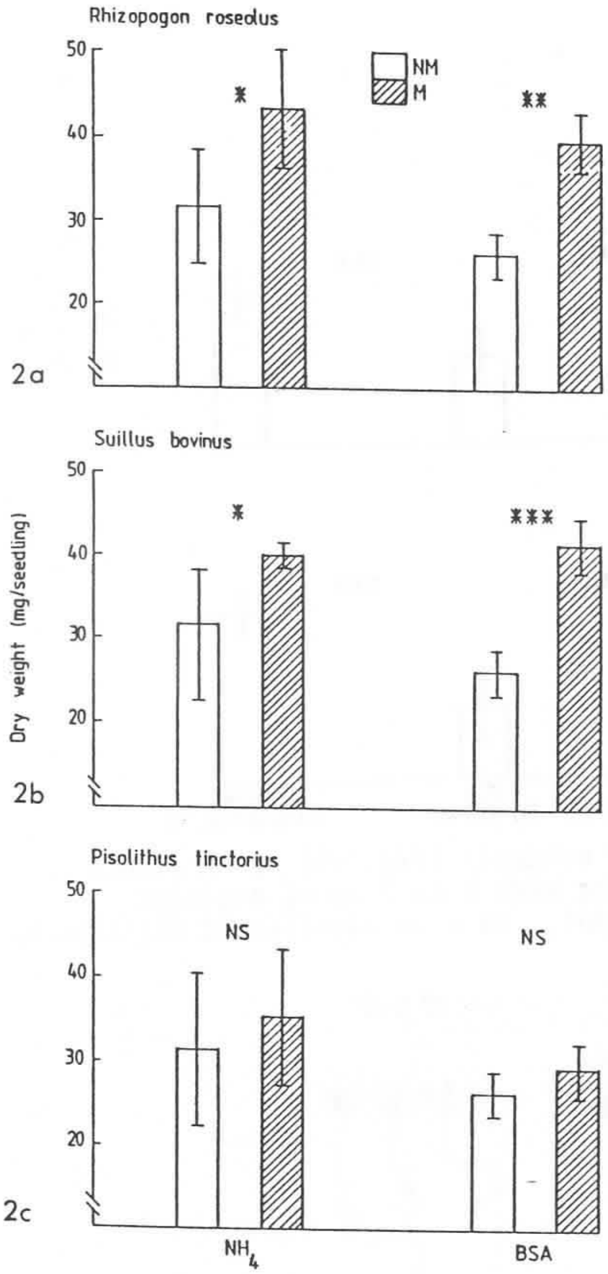

Figure 2. Dry weights of P. contorta after 60 d with R. roseolus (Fig. 2a) S. bovinus (Fig. 2b), P. tinctorius (Fig. 2c) or without mycorrhiza (NM), on (NH_4^+) or BSA as N sources. +++ = $P < 0.001$ NS= no significant difference. Bars show 95% confidence limits.

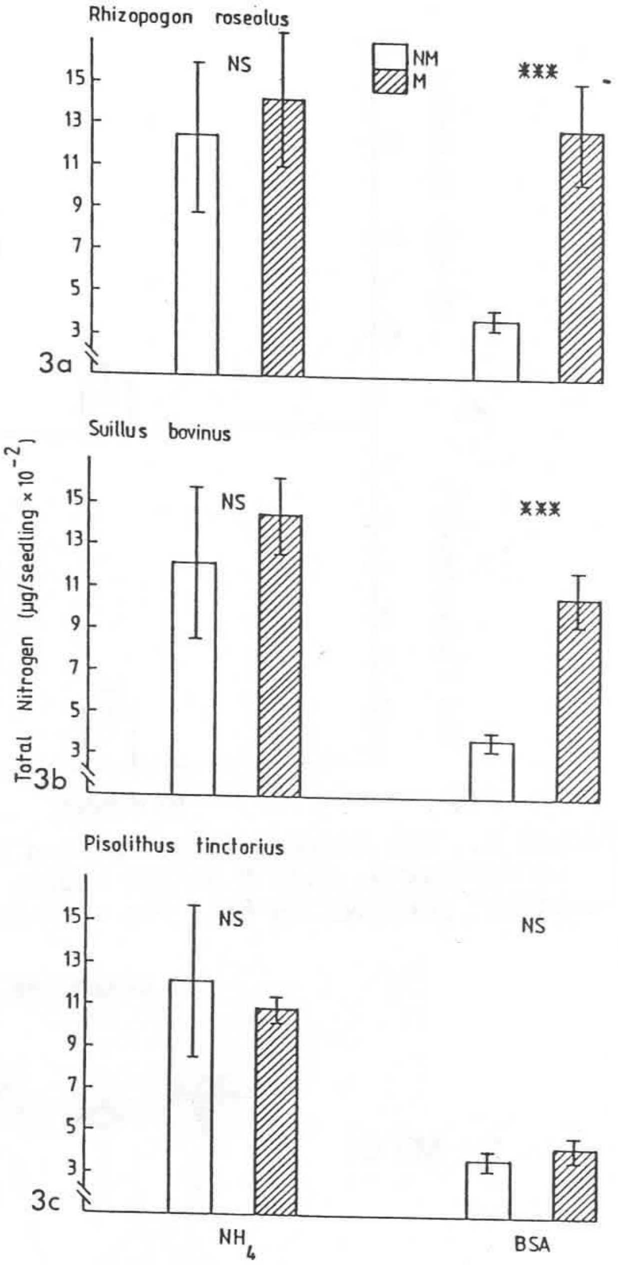

Figure 3. Nitrogen contents of P. contorta. Other details as in Fig. 2.

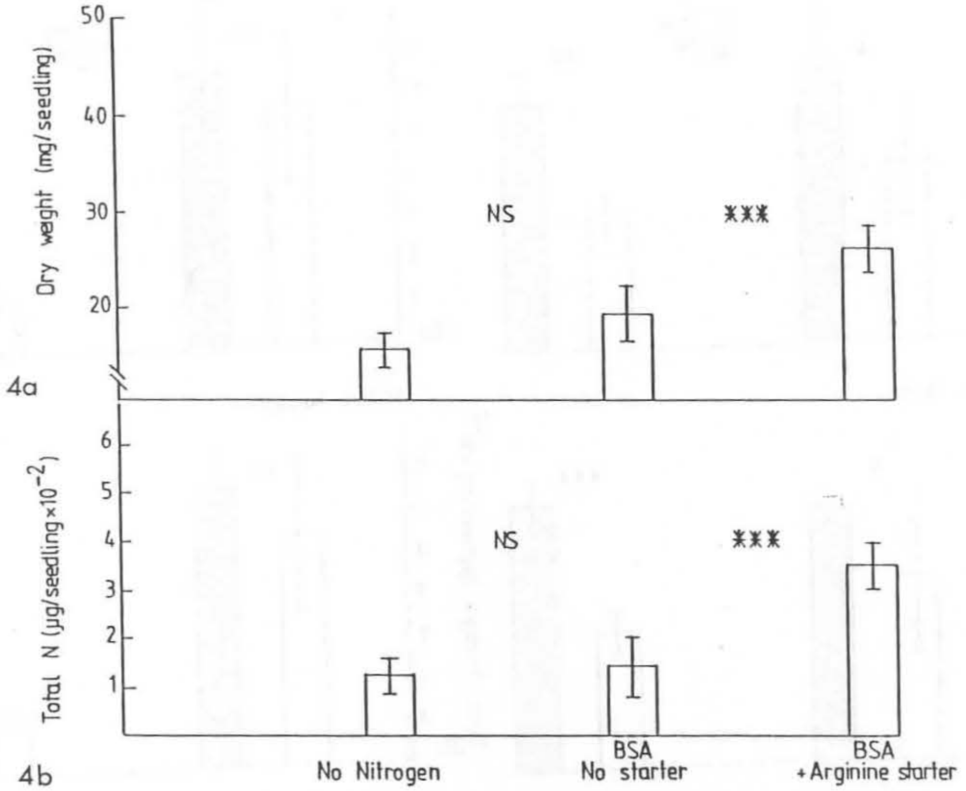

Figure 4. Dry weights (Fig. 4a) and N contents (Fig. 4b) of NM plants grown without N, with N as BSA alone, or with N as 1 mg of arginine 'starter' followed by BSA. +++ = $P < 0.001$, NS = no significant difference.

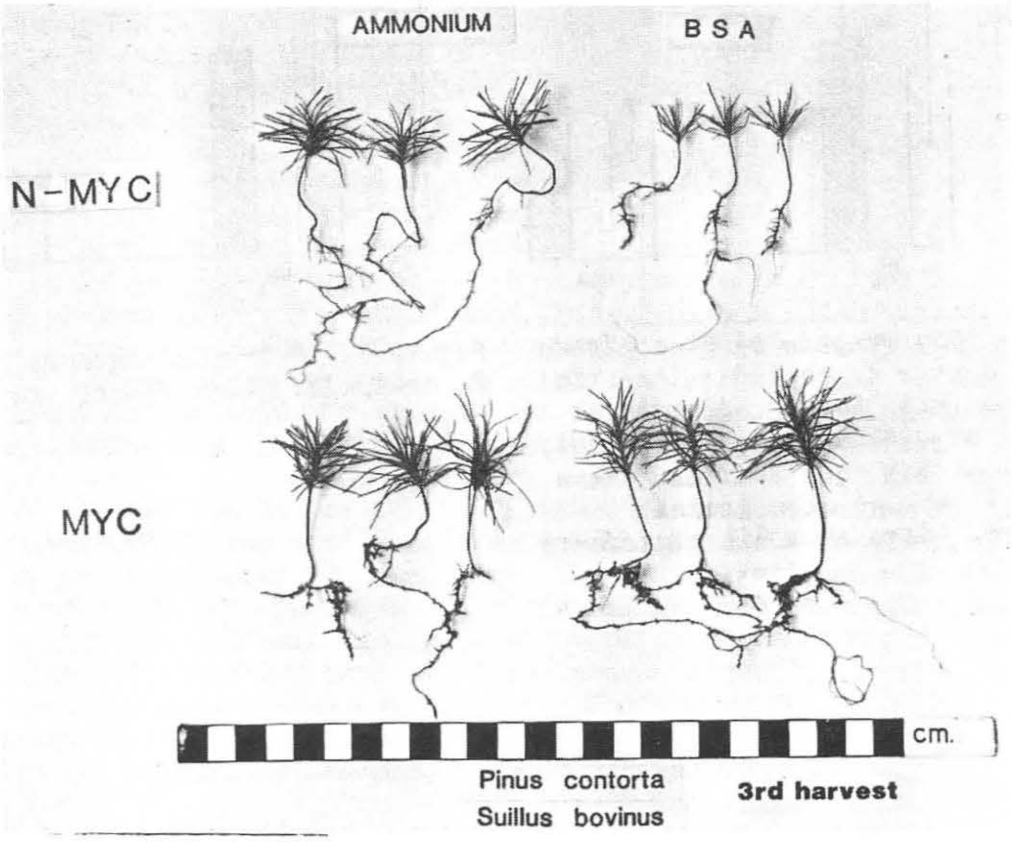

Figure 5. Plants grown with S. bovinus (MYC) or without mycorrhiza (N-MYC) with NH_4^+ or BSA as N source.

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